Correlation of Apolipoprotein E Gene Polymorphism to Serum Lipid Concentrations in Healthy Thais†

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Abstract

This study determined the genotype distribution of apolipoprotein E (apo E) gene and its relation to serum lipids in 217 healthy Thais consisting of 79 males and 138 females. Serum total cholesterol (TC), HDL-cholesterol (HDL-C) and triglyceride (TG) concentrations were determined by enzymatic-colorimetric methods, while serum LDL-cholesterol (LDL-C) levels were calculated using Friedewald formula. Apo E genotypes were determined by PCR-RFLP. Out of 217 subjects, apo E genotype frequencies were 5.5 per cent for E2/E2, 12.4 per cent for E2/E3, 81.1 per cent for E3/E3 and 0.9 per cent for E4/E4. In men, advancing age was associated with increased serum TC ($r = 0.28$, $P < 0.05$) and LDL-C ($r = 0.27$, $P < 0.01$). Subjects having the E2 allele had lower TC ($r = -0.27$, $P < 0.05$) and LDL-C. ($r = -0.25$, $P < 0.05$). Age and apo E genotypes were not associated with HDL-C and TG in men. In women, increasing age was related to higher serum TC ($r = 0.45$, $P<0.001$), LDL-C ($r = 0.44$, $P < 0.001$), TG ($r = 0.40$, $P < 0.001$) and lower HDL-C ($r = -0.36$, $P < 0.001$). The presence of E2 allele was related to lower TC ($r = -0.24$, $P < 0.001$), LDL-C ($r = -0.26$, $P < 0.001$), TG ($r = -0.15$, $P < 0.05$) and higher HDL-C ($r = 0.20$, $P < 0.01$) independent of age and menopausal status.We concluded that the ε4 allele of apo E gene is rare in Thais. The presence of the ε2 allele is associated with a more favorable lipid profile and there is a sexual dimorphism concerning the effect of apo E genotype on serum HDL-C and TG.

Key word : Apolipoprotein E, Genetic Polymorphism, Serum Lipids, Atherosclerosis

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Apolipoprotein E (apo E) is a major protein component of lipoprotein and plays an important role in the hepatic lipoprotein uptake and reverse cholesterol transport. Circulating apo E is heterogeneous in terms of protein structure and common apo E isoforms include E2, E3 and E4 which are coded by the ε2, ε3 and ε4 alleles at apo E gene locus, respectively. Apo E polymorphism has been associated with serum lipid concentrations in a number of populations[1,2]. Besides association with serum lipid levels, apo E polymorphism has been demonstrated to be related to cardiovascular diseases[3]. It is well known that gender and age also affect serum lipid concentrations. With advancing age, serum lipid profile becomes more atherogenic[4]. Moreover, the more favorable lipid profiles, when compared to men, in younger women tend to disappear after menopause[5]. It is unclear how the genetic effect of apo E polymorphism may interact with gender and age-related factors to determine serum lipid profiles since most studies to date did not examine such interactions concurrently.

In the present study, we determined the genotype distribution of apo E and its relation to serum lipids in healthy Thais. The gender and age-related effects were also addressed concurrently to assess the importance of apo E and its interactions with age and gender in the determination of serum lipids in Thais.

MATERIAL AND METHOD

Subjects

Seventy nine nondiabetic men and 138 nondiabetic women residing in the Bangkok Metropolitan area were recruited by leaflets or direct contact. All were found to be healthy by medical history-taking and complete physical examination.

All subjects gave informed consent and the study was approved by the Ethical Clearance Committee on Human Rights Related to Researches Involving Human Subjects of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University.

Laboratory Assessments

Fasting blood samples were obtained from subjects between 8.00 and 10.00 am. Serum samples were frozen at -20°C until measurement. Serum total cholesterol (TC), HDL-cholesterol (HDL-C) and triglyceride (TG) concentrations were determined by enzymatic-colorimetric method[6-8], while serum LDL-cholesterol (LDL-C) levels were calculated using Friedewald formula[9].

Determination of Apo E genotypes

Apo E genotypes were determined by a previously reported method[10] with some modifications. Genomic DNA (0.1 μg) was amplified in 50 μl of 20 mM Tris-HCl, pH 8.0 and 160 μM of each of the four deoxyribonucleotides. One unit of Taq polymerase and 0.2 μM of each of the oligonuccleotide primers were used for the reaction. Polymerase chain reaction was performed through 40 cycles by the following steps: denaturation at 94°C for 30 seconds, annealing at 65°C for 30 seconds and extension at 30°C for 1 minute. After amplication, the PCR product was digested with HhaI restriction endonuclease and resolved in 5 per cent Metaphor agarose gel (FMC Bio Product, USA) with ethidium bromide staining.

Statistical Analyses

Association between serum lipids and apo E alleles was analyzed using Pearson's correlation. The presence of ε2 allele was numerically coded as 1 while the absence of the allele was coded as 2. Assessments of difference between 2 categories were performed by Mann-Whitney U test. Stepwise multiple linear regression was used to determine the concurrent effects of multiple variables on serum lipids. Data were presented as mean ± SEM.

RESULTS

Clinical characteristics and serum lipid concentration in male and female subjects are shown in Table 1. As demonstrated in Table 2. advancing age was associated with increased serum TC and LDL-C in men while HDL-C and TG were not related to increasing age. In women, increasing age was also related to higher serum TC and LDL-C. However, unlike the findings in men, increasing age in women was associated with higher TG and lower HDL-C. When categorizing the subjects into age groups, it was found that women had significantly higher HDL-C than men in all age groups while the lower TG compared to men in younger women no longer persisted when age was greater than 60 years (Table 3). No gender-related difference in TC or LDL-C was demonstrated in any age groups.

The distribution of apo E genotypes is shown in Table 4. The ε4 allele was rare in our population. There was no difference in genotypes distribution between men and women. Men with ε2

Table 1. Clinical characteristics and serum lipid concentrations in male and female subjects.

	Males (n = 79)	Females (n = 138)
Age (year)	51.8 ± 1.8	50.8 ± 1.2
Body weight (kg)	66.0 ± 1.3	54.9 ± 0.7
Total cholesterol (mg/dl)	220.4 ± 4.4	224.3 ± 3.5
LDL-cholesterol (mg/dl)	160.8 ± 4.3	162.5 ± 3.4
HDL-cholesterol (mg/dl)	31.8 ± 1.1	40.9 ± 0.8
Triglyceride (mg/dl)	138.9 ± 8.4	104.1 ± 4.4

Data are presented as mean ± SEM

Table 2. Correlations between serum lipid concentrations and age in males and females.

	Males		Females	
	r	p	r	p
Total cholesterol (mg/dl)	0.28	< 0.05	0.45	< 0.001
LDL-cholesterol (mg/dl)	0.29	< 0.05	0.45	< 0.001
HDL-cholesterol (mg/dl)	-0.07	NS	-0.36	< 0.001
Triglyceride (mg/dl)	-0.02	NS	0.41	< 0.001

Table 3. Comparison of serum lipid concentrations between males and females in different age groups.

	Males	Females	p
Age < 40 years	n = 19	n = 29	
Total cholesterol (mg/dl)	192.8 ± 9.2	200.6 ± 6.6	NS
LDL-cholesterol (mg/dl)	135.1 ± 8.7	137.7 ± 6.2	NS
HDL-cholesterol (mg/dl)	33.4 ± 3.1	47.1 ± 1.3	< 0.001
Triglyceride (mg/dl)	121.9 ± 19.4	78.5 ± 6.4	< 0.05
Age 40.0 – 59.9 years	n = 31	n = 71	
Total cholesterol (mg/dl)	232.4 ± 6.2	220.9 ± 4.4	NS
LDL-cholesterol (mg/dl)	171.7 ± 6.7	160.9 ± 4.3	NS
HDL-cholesterol (mg/dl)	29.4 ± 1.3	40.5 ± 1.1	< 0.001
Triglyceride (mg/dl)	156.4 ± 14.6	97.5 ± 5.5	< 0.001
Age ≥ 60 years	n = 31	n = 42	
Total cholesterol (mg/dl)	229.1 ± 6.4	245.1 ± 6.5	NS
LDL-cholesterol (mg/dl)	170.0 ± 6.3	181.4 ± 8.1	NS
HDL-cholesterol (mg/dl)	33.5 ± 1.3	37.7 ± 1.4	< 0.05
Triglyceride (mg/dl)	128.2 ± 9.3	130.2 ± 9.0	NS

Data are presented as mean ± SEM

Table 4. Distribution of apo E genotypes in males and females.

	Apo E Genotype					
	ε2ε2	ε2ε3	ε2ε4	ε3ε3	ε3ε4	ε4ε4
Males (n = 79)	4 (5.1%)	13 (16.5%)	0	62 (78.5%)	0	0
Females (n = 138)	8 (5.8%)	14 (10.1%)	0	114 (82.6%)	0	2 (1.4%)
Total (n = 217)	12 (5.5%)	27 (12.4%)	0	176 (81.1%)	0	2 (0.9%)

Table 5. Comparison of serum lipid concentrations in males with and without ε2 allele.

	ε2 present (n = 17)	ε2 absent (n = 64)	p
Total cholesterol (mg/dl)	200.6 ± 10.7	225.9 ± 4.5	< 0.05
LDL-cholesterol (mg/dl)	142.8 ± 10.0	165.9 ± 4.7	< 0.05
HDL- cholesterol (mg/dl)	31.9 ± 1.9	31.7 ± 1.2	NS
Triglyceride (mg/dl)	129.8 ± 12.5	141.5 ± 10.1	NS

Data are presented as mean ± SEM

Table 6. Comparison of serum lipid concentrations in premenopausal and postmenopausal females with and without ε2 allele.

	ε2 present	ε2 absent	p
Premenopausal females	n = 11	n = 46	
Total cholesterol (mg/dl)	180.9 ± 10.4	208.3 ± 4.2	< 0.05
LDL-cholesterol (mg/dl)	121.6 ± 10.1	147.2 ± 4.3	< 0.05
HDL-cholesterol (mg/dl)	45.5 ± 2.3	44.3 ± 1.4	NS
Triglyceride (mg/dl)	69.0 ± 9.6	84.0 ± 4.8	< 0.05
Postmenopausal females	n = 11	n = 70	
Total cholesterol (mg/dl)	219.0 ± 11.7	242.5 ± 4.9	< 0.05
LDL-cholesterol (mg/dl)	153.6 ± 11.7	180.5 ± 4.5	< 0.05
HDL-cholesterol (mg/dl)	45.5 ± 2.3	37.3 ± 1.0	< 0.01
Triglyceride (mg/dl)	99.3 ± 10.1	123.5 ± 7.1	NS

Data are presented as mean ± SEM

allele had lower TC and LDL-C (Table 5). However, the presence of ε2 allele did not affect HDL-C and TG in men. Similar to the findings in men, pre- and postmenopausal women with ε2 allele had lower TC and LDL-C (Table 6). Nevertheless, pre- and postmenopausal women with ε2 allele also had lower TG and higher HDL-C, respectively.

Using stepwise regression analysis to assess the simultaneous effects of advancing age and apo E genotype on serum lipids, it was found that advancing age and the absence of ε2 allele were associated with higher TC and LDL-C in men. No effect on HDL-C and TG was demonstrated (Table 7). In women, advancing age and the absence of ε2 allele were associated with higher TC, LDL-C, TG and lower HDL-C (Table 7). Menopausal status did not affect serum lipids after controlling for age.

DISCUSSION

Apo E polymorphism display ethnic variations especially for the frequency of E4 allele. In Caucasians, the proportion of the population harboring E4 allele is in the 10 - 20 per cent range. In contrast, the prevalence of E4 allele in Asians is low. In Chinese, the prevalence of E4 allele has been reported to be 4.9 per cent[11] while it is 11.1 per cent in Japanese[12]. In the present study in Thais, low prevalence of E4 allele was also demonstrated. The reason for the different gene distribution is unclear. Nevertheless, it has been suggested that different distribution of apo E polymorphism may be accountable for the variation in the prevalence of atherosclerosis among different populations[13,14]. Apo E polymorphism was associated with higher mortality from cardiovascular disease in elderly women[15] and early atherosclerosis as assessed by carotid intimal thickness[16] and the initiation and progression of atherosclerosis[17]. Changes in lipid profile and the size of LDL particles[18] may mediate the effect of apo E polymorphism on coronary heart disease. Our present findings concerning the influence of apo E genotypes on serum lipids are in keeping with other previous

Table 7. Standardized coefficients of regression from stepwise multiple linear regression analyses of the relation between age, apo E polymorphism and serum lipids in males and females.

	Age		ε2 status	
	ß	p	ß	p
Males				
Total cholesterol (mg/dl)	0.28	< 0.05	-0.27	< 0.05
LDL-cholesterol (mg/dl)	0.27	< 0.01	-0.25	< 0.05
HDL-cholesterol (mg/dl)	-	NS	-	NS
Triglyceride (mg/dl)	-	NS	-	NS
Females				
Total cholesterol (mg/dl)	0.45	< 0.001	-0.24	< 0.001
LDL-cholesterol (mg/dl)	0.44	< 0.001	-0.26	< 0.001
HDL-cholesterol (mg/dl)	-0.36	< 0.001	0.20	< 0.05
Triglyceride (mg/dl)	0.40	< 0.001	-0.15	< 0.05

studies showing that E2 allele is more favorable in terms of lipid profiles[1,2] while E4 allele is the least favorable. The association between apo E and serum lipids is evident in children as young as 3 years old. However, the effect is not apparent in the newborn suggesting that environmental factors are needed for apo E polymorphism to exert its effect[19].

It is well established that aging is associated with changes in serum lipids, particularly an increase in LDL-C and TG and decrease in HDL-C as demonstrated in the present study. It has been reported that there is an interaction of aging and genotype on serum lipids[20,21]. However, It is of note that the effect of apo E polymorphism on serum lipid concentrations is partly gender-related such that apo E polymorphism was related to variation in serum HDL-C and TG only in women. The reason for the gender-related modulation is unclear. Few studies have addressed the gender-related difference in the effect of apo E on serum lipids. Similar to our findings, a study in American Indians demonstrated a gender difference such that the relation between apo E and TG was stronger in postmenopausal women compared to men[22]. Estrogen has a number of effects on serum lipids. For example, it has been shown that estrogen decreases LDL-C, increases HDL-C and may decrease TG levels. The mechanisms by which estrogen affect serum lipids include stimulating hepatic VLDL uptake, induction of hepatic LDL receptors and LDL uptake. It is likely that there may be an interaction between estrogen and apo E systems on serum lipids. In fact, estrogen replacement has been demonstrated to decrease serum apo E levels[23, 24]. Although there were studies showing different responses to lipid-lowering agents such as bezafibrate and pravastatin among subjects with different apo E polymorphism, data on such an effect of estrogen is not available.

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ความสัมพันธ์ระหว่างโพลิมอฟีซั่มของยีนอะโปไลโปโปรตีนอีและระดับไขมันในเลือดในคนไทย

สุวรรณี ชั้นประเสริฐโยธิน, วท.ม., บุญส่ง องค์พิพัฒนกุล, พ.บ.**, รัชตะ รัชตะนาวิน, พ.บ.**, นพวรรณ เปียซื่อ, วท.ม.**, ละออ ชัยลือกิจ, วท.ม.**, กอบชัย พัววิไล, พ.บ.***

จากการศึกษาความถี่ของยีนอะโป อี ในคนไทยสุขภาพสมบูรณ์ จำนวน 217 คน แบ่งเป็นชาย 79 คน และหญิง 138 คน พบว่ามีลักษณะของยีน E2/E2, E2/E3, E3/E3 และ E4/E4 เท่ากับ 5.5%, 12.4%, 81.8% และ 0.9% ตามลำดับ ในชายพบว่าอายุมีความสัมพันธ์เชิงบวกกับระดับโคเลสเตอรอล และระดับแอลดีแอลโคเลสเตอรอล แต่ถ้ามีลักษณะของยีน E2 ร่วมด้วย จะทำให้ระดับไตรกลีเซอร์ไรด์และระดับแอลดีแอลโคเลสเตอรอลลดลง แต่จะไม่มีความสัมพันธ์กับระดับเอชดีแอลโคเลสเตอรอล ในหญิงพบว่าอายุมีความสัมพันธ์เชิงบวกกับระดับโคเลสเตอรอล, ไตรกลีเซอร์ไรด์, และแอลดีแอลโคเลสเตอรอล แต่มีความสัมพันธ์เชิงลบกับระดับเอชดีแอลโคเลสเตอรอล ถ้ามีลักษณะของยีน E2 ร่วมด้วยก็จะทำให้ระดับโคเลสเตอรอล, ไตรกลีเซอร์ไรด์, และแอลดีแอลโคเลสเตอรอลลดลง ส่วนระดับเอชดีแอลโคเลสเตอรอลจะเพิ่มขึ้น ในประชากรไทยจะพบลักษณะของยีน E4 ได้น้อย และยีน E2 จะทำให้ระดับไขมันดีขึ้น อะโป อี ยีน มีผลต่อระดับเอชดีแอลโคเลสเตอรอลและไตรกลีเซอร์ไรด์ต่างกันในเพศที่ต่างกัน

คำสำคัญ : อะโปไลโปโปรตีนอี, ความหลากหลายทางพันธุกรรม, ระดับไขมัน, หลอดเลือดแดงแข็ง

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